CytoSorbents Corporation

CytoSorb® REduction of FREe Hemoglobin/Acute Kidney Injury (AKI) during Cardiac Surgery

REFRESH II

2017-001

Statistical Analysis Plan

Version 1, 05JUN2022

Version History

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1.0	05JUN2022	Lisa Thackeray/ Sr. Principal Biostatistician Peter Shores/ Sr. Biostatistician	Initial Release

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Table of Contents

1 Introduction

This statistical analysis plan (SAP) describes the planned statistical methods to be used during the reporting and analysis of data collected under the Clinical Investigation Protocol *CytoSorb® REduction of FREe Hemoglobin/Acute Kidney Injury (AKI) during Cardiac Surgery* (REFRESH II). The study was stopped early at the discretion of the sponsor and analyses described herein reflect those that will be performed for the final report and may not reflect those stated in the CIP.

This SAP should be read in conjunction with the study clinical investigation plan (CIP) and case report forms (CRFs). The SAP has been developed with respect to the most updated version of the REFRESH II Protocol Version F, 15SEP2020.

Applicable Documents:

Document Number, Version	*Document Title*
2017-001, Version F	*CytoSorb® REduction of FREe Hemoglobin/Acute Kidney Injury (AKI) during Cardiac Surgery* (REFRESH II)

2 Abbreviations

Abbreviation/Term	*Definition*
AE	Adverse Event
AKI	Acute kidney injury
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BMI	Body mass index
C3a	Serum complement component C3a
C5a	Serum complement component C5a
CABG	Coronary artery bypass graft
CIP	Clinical investigational plan
CP	Conditional probability
CPB	Cardiopulmonary bypass
CRF	Case Report Form
eGFR	Estimated glomerular filtration rate

Abbreviation/Term	*Definition*
FCE	Field Clinical Engineer
GSD	Group sequential design
HCA	Hypothermic Circulatory Arrest
HMGB1	High mobility group box protein 1
IA	Interim Analysis
ICU	Intensive care unit
IGFBP7	Insulin-like growth factor binding protein 7
INR	International normalized ratio
ITT	Intent-to-Treat
KDIGO	Kidney Disease: Improving Global Outcome group
LVEF	Left ventricular ejection fraction
MedDRA SOC	MedDRA System organ class
mITT	Modified Intent-to-Treat
NGAL	Neutrophil gelatinase-associated lipocalin
NYHA	New York Heart Association
OR	Operating room
$paC0_2$	Partial pressure of carbon dioxide
paO_2	Partial pressure of oxygen
pfHb	Plasma free hemoglobin
PP	Per Protocol
PT	Prothrombin time
RBC	Red blood cell
SAE	Serious adverse event
SI	Standard units
SoC	Standard of care
SOFA	Sequential Organ Failure Assessment Score
SSR	Sample size re-estimation
TIMP-2	Tissue inhibitors of metalloproteinases

Abbreviation/Term	*Definition*
UADE	Unanticipated adverse device effect
WBC	White Blood Cell

3 Study Objectives

The primary objective of the study is to evaluate the safety and performance of the CytoSorb® device to decrease the incidence or severity of AKI in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing complex cardiac surgery. The study hypothesis is that, for patients at increased risk for AKI based upon use of the Cleveland Clinic Score for stratification of the risk of cardiac surgery associated AKI (CSA-AKI), the intraoperative usage of the CytoSorb® device in addition to the standard of care in a bypass circuit during cardiopulmonary bypass will decrease the overall incidence and/or proportion of AKI by stage as defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury.

The study intent is that the intraoperative use of the CytoSorb® device, in conjunction with a standard of care CPB circuit will decrease the incidence or severity of AKI per the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines definition of acute kidney injury as compared to a standard of care control population during the first 48 hours post cardiac surgery.

4 Study Design

The REFRESH II study is a prospective, multi-center, randomized, pivotal, double-blinded clinical study. Subjects will be randomized in a 1:1 ratio to either standard of care (SoC) alone or standard of care and treatment with the CytoSorb® device.

To ensure proper training and competence related to integration of the investigational study device at all sites a roll-in approach was employed where the first two enrolled subjects from each site were used for training. All subjects enrolled as roll-in cases were treated with the CytoSorb® device and followed per protocol.

The initial design included interim analyses with the potential of (NON-BINDING) early stopping for an efficacy or futility conclusion, and an option to increase sample size (SSR, i.e., Sample Size Re-estimation).

4.1 Study Design Changes

The study was stopped early at the discretion of the sponsor, prior to any interim analyses being performed. Total enrollment, including roll-ins reached 151 subjects of the planned 420. Analyses described within this SAP reflect those that will be performed for the final report that includes results

from the 151 patients and may not reflect those originally planned for the total study population as stated in the CIP.

4.2 Randomization

Subjects will be randomized prior to the start of CPB surgery (within 5 business days prior to and including day of procedure), but only after the subject provides written informed consent and subject eligibility criteria is confirmed. Randomization will be stratified by study center and intended use of HCA as a surgical technique during the procedure, with a 1:1 allocation ratio (SoC+CytoSorb®:SoC alone). Randomized treatment will be assigned from the electronic database.

4.3 Blinding

See Section 3.6 of the CIP for additional details on Maintaining the Study Blind and Unblinding Procedures.

5 Sample Size Determination

The REFRESH II study hypothesis is that, for patients at increased risk for AKI based upon use of the Cleveland Clinic Score for stratification of the risk of cardiac surgery associated AKI (CSA-AKI), the intra-operative usage of the CytoSorb® device in addition to the standard of care in a bypass circuit during cardiopulmonary bypass will decrease the overall incidence and/or proportion of acute kidney injury (AKI) by stage as defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline definition of acute kidney injury. The AKI Stage is scored as 0, 1, 2 or 3. AKI Stage of 0 indicates no AKI, and AKI stages 1, 2 and 3 indicate mild, moderate, and severe AKI, respectively. Success will be defined by demonstrating a shift in distribution toward overall less severe AKI or fewer incidences of AKI in the CytoSorb® population. For full explanation of sample size determination, refer to 2017-001, Version F section 11.2.

6 Statistical Analyses

6.1 General Considerations

Except where otherwise specified, the following general principles apply to the planned statistical analyses. All statistical analysis will be conducted using SAS version 9.4 or later (SAS Institute Inc., Cary, NC) and/or the R software language.

6.1.1 Descriptive Statistics

Tabulations will be produced for appropriate demographic, baseline, efficacy, safety, and other parameters by treatment group and overall, with Roll-in subjects presented in parallel unless specified otherwise.

Continuous data will be summarized with mean, standard deviation, median, interquartile range, minimum, and maximum. Categorical variables will be summarized with frequency counts and percentages.

6.1.2 Study Day

Study day 0 is the date of the index procedure. Day in study will be calculated relative to the index procedure as follows:

Study Day = Assessment Date – Index Procedure Date

For each subject, duration in study will be based on last study contact date which is the latest date of all follow-up visits, assessments, adverse event onset or resolution, and study exit including date of death.

Duration variables will be calculated as follows:

Duration Days = End Date – Start Date

6.1.3 Visit Windows

Unless otherwise specified, visit based assessments will be analyzed for each analysis time point according to the nominal visit entered in the Case Report Form (CRF) regardless of if it is out of window.

6.1.4 Statistical Significance

Unless otherwise specified, hypothesis testing will be performed at the two-sided 0.05 significance level. P-values will be rounded to three decimal places. If a p-value is less than 0.001 it will be reported as “<0.001”. If a p-value is greater than 0.999, it will be reported as “>0.999”.

6.2 Analysis Populations

The following subject populations will be evaluated and used for presentation and analysis of the data:

- Intent-to-Treat (ITT): All randomized patients.

  Safety (SAF): Comprised of all roll-in and randomized subjects in whom treatment was attempted. Safety analysis will be performed on this population. Patients will be summarized according to the actual treatment received, with Roll-in subjects presented in parallel.

6.3 Handling of Missing Data

All attempts will be made to limit the amount of missing data. Unless otherwise specified, no attempt will be made to impute missing data. If a data point is missing, that data point will not contribute to that portion of the analysis.

6.4 Subject Disposition

A summary of subject disposition will be tabulated for all subjects by treatment group and overall, including:

- Number of screened patients
- Number of patients who failed screening
- Number of subjects enrolled
- Number of patients randomized
- Number of patients treated
- Number of patients who completed treatment
- Number of patients who discontinued treatment and the reasons for treatment discontinuation
- Number of patients who completed study
- Number of patients who discontinued study participation and the reasons for discontinuation from the study.

The number of patients included in each analysis population will also be summarized.

6.5 Demographics and Baseline Characteristics

Baseline characteristics will include standard demography (e.g., age, gender, ethnicity, race, weight, height, BMI), NYHA classification, smoking history, number of blocked vessels, and extent of coronary heart disease. Medical history will include information on pre-existing/prior AKI, cardiovascular disease, peripheral vascular disease, diabetes mellitus, hypertension, hyperlipidemia, obesity, prior TIA or stroke, etc.

Demographic and baseline characteristics will be summarized for the ITT population using descriptive statistics for each treatment group and overall, with Roll-in subjects presented in parallel.

Medical history by MedDRA system organ class (SOC) and preferred name will be summarized by treatment group and overall. For each subject, multiple medical histories of the same MedDRA SOC or preferred name will be counted only once within each MedDRA SOC and preferred name. Medical histories will be sorted alphabetically by MedDRA SOC and preferred name.

Previous procedure will be summarized as the number and percentage of subjects by procedure category, treatment group and overall, with Roll-in subjects presented in parallel.

6.6 Procedural Data

Procedural data will include procedure type, valves involved, number of grafts, valve involvement, evidence of cerebral perfusion and type (if applicable), hemoconcentrator use, heparin use, lowest core body temperature, and procedural durations (i.e., total operative time, total CPB time, cross clamp duration, etc.).

Data will be summarized for the ITT, and safety population using descriptive statistics for each treatment group and overall, with Roll-in subjects presented in parallel.

6.7 Analysis of Study Endpoints

6.7.1 Primary Efficacy Endpoints

The primary effectiveness endpoint is the incidence or severity of AKI (i.e., incidence of AKI and proportion of AKI by stage) in the first 48 hours after surgery when used intraoperatively with cardiopulmonary bypass (CPB) in subjects undergoing cardiac surgery as defined by the KDIGO clinical practice guideline through 48 hours post-cardiac surgery.

6.7.1.1 Primary Analysis

AKI severity (stage 0, 1, 2, 3) based on creatinine measured at CPB, 24-hours post-CPB, and 48-hours post-CPB will be summarized using descriptive statistics. Summaries will be provided for the ITT and Roll-ins. Data will be displayed by treatment group and overall, with Roll-ins presented in parallel. AKI stage is based on the 'worst' criteria being met and subjects included in each severity level will be mutually exclusive across levels.

The number and proportion of subjects will be given for the following severity breakdowns:

- Stage 0 (no AKI)
- Stage 1 (mild AKI)
    - Serum creatinine increase of 1.5-1.9 times baseline, or
    - Serum creatinine increase of ≥0.3mg/dL over baseline, or
    - urine output < 0.5 mL/kg/h for 6 h
- Stage 2 (moderate AKI)
    - Serum creatinine increase of 2.0-2.9 times baseline, or
    - urine output < 0.5 mL/kg/h for ≥ 12 h
- Stage 3 (severe AKI)
    - Serum creatinine ≥3 times baseline, or
    - Serum creatinine increase to ≥4.0mg/dL, or
    - Initiation of renal replacement therapy, or
    - urine output < 0.3 mL/kg/h for ≥ 24 h, or
    - anuria for ≥ 12 h
- Stage 1, 2, or 3
- Not reported

Comparisons between SoC and SoC+CytoSorb® will be made using Fisher's exact tests. Statistical comparisons will be done for the ITT population only.

6.7.2 Secondary Efficacy Endpoints

All secondary efficacy analyses will be conducted using the ITT population

6.7.2.1 Health Economic Summary

Health economic parameters will be evaluated and include the following:

- Duration of hospital stay post-surgery = Date of discharge from hospital – Date of surgery completion + 1

- Duration of ICU stay post-surgery = Date of discharge from ICU – Date of admission to ICU/date of surgery completion + 1
- Duration of renal replacement therapy post-surgery= End date of therapy - Start date of therapy (post-surgery) + 1
- Rate of vasopressor medication use post-surgery
- Duration of ventilator use post-surgery = Ventilation end date – date of surgery completion + 1

Health economic data will be summarized using descriptive statistics by treatment group and overall, with Roll-ins presented in parallel.

6.8 Safety Analyses

Safety endpoints will be based on the Safety population.

For purposes of analyses relatedness to device or procedure will be defined as follows:

- An AE will be considered surgical procedure related if the relationship is definite, probable, possible, unrelated, or unknown.
- An AE will be considered investigational device related if the relationship is definite, probable, possible, unrelated, or unknown.

By-subject listings will be provided for all adverse events.

6.8.1 Primary Safety Endpoint

The primary safety endpoint of this trial is to evaluate the product safety profile through the assessment of device related adverse events during the study period.

There is no formal hypothesis associated with the primary safety endpoint. Device related adverse events will be tabulated with the number of events and subjects with event for each MedDRA (Version 21.0) SOC/preferred term and overall. Rates will be reported as the number of subjects who experience at least one event out of the total number of subjects.

6.8.2 Secondary Safety Endpoints

6.8.2.1 Procedure-related Adverse Events

The incidence and severity of procedure-related AEs will be assessed by tabulating the number of events and subjects with event for each event type and overall. Rates will be reported as the number of subjects who experience at least one event out of the total number of subjects. Data will also be tabulated for serious procedure-related AEs. Data will be summarized on the safety population.

6.8.2.2 Serious Device-related Adverse Events

The serious device-related adverse events in the study will be summarized similarly to the primary safety endpoint.

6.8.2.3 Unanticipated Adverse Device Effects

The incidence and severity of all unanticipated adverse device effects will be summarized similarly to the primary safety endpoint.

6.8.2.4 Change in Vital Signs

Vital signs include body temperature, heart rate, respiration rate, pulse oximetry, and blood pressure. The observed value of vital signs will be summarized for each visit, starting with admission to the ICU post-procedure, and then daily beginning on Day 1 post-procedure.

The change in vital signs between baseline and Day 1 post-procedure will also be summarized.

6.8.2.5 Change in Laboratory Assessments

Clinical laboratory evaluations include:

- Hematology: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, platelet count
- Serum Chemistry: sodium, potassium, chloride, bicarbonate (CO2), BUN (urea), creatinine, serum glucose, calcium, AST (SGOT), ALT (SGPT), alkaline phosphatase, total bilirubin, albumin, total protein
- Coagulation: aPTT, INR, PT, fibrinogen

Clinical laboratory values will be expressed using the standard units (SI).

In the event of repeat values within the same analysis visit, the first measurement at that visit was used.

For hematology and serum chemistry, the observed value and change from baseline will be summarized by visit for continuous clinical laboratory parameter. Shift tables will be provided to summarize count

and percent of patients with changes from baseline normal range status (below, within, above) to on/post-treatment normal range status.

6.8.3 Arterial Blood Gas Measurements

Arterial blood gas measurements (PaO^2, PaCO^2, pH, HCO^3) observed value and change from baseline will be summarized using descriptive statistics at each time point by treatment group and overall, with Roll-ins presented in parallel for patients in the safety population.

FIO2 values were not originally required per protocol. At the request of the DMC, a new field was added to the EDC system to retrospectively collect FIO2 values. Observed values for FIO2 and PaO2/FIO2 Ratio will be summarized using descriptive statistics at each time point by treatment group and overall, with Roll-ins presented in parallel for patients in the safety population.

6.8.4 Blood Product Transfusions

Blood product transfusions are broken into time intervals including intra-operative, post-operative on the day of surgery, day 1 post-surgery, and day 2+ post-surgery (through the end of hospitalization). Data on blood product transfusions will be summarized using descriptive statistics by treatment group and overall, with Roll-ins presented in parallel for patients in the safety population

6.8.5 Additional Post-surgical Care Unit Assessments

Additional post-surgical Care Unit assessments (contributing to the overall health economic summary) include the following through hospital discharge or post-surgical Day 7 (whichever is first): SOFA, vasopressor requirements, requirement for mechanical support, and renal replacement requirements. Data will be summarized using descriptive statistics for each assessment by treatment group and overall, with Roll-ins presented in parallel for patients in the safety population

6.8.6 Additional Safety

The total adverse events in the study will be summarized similarly to the primary safety endpoint. A summary will be presented by seriousness, severity, relationship to surgical procedure, relationship to investigational device, and relationship to non-investigational device will be provided using counts and percentage.

Non-serious adverse events will be tabulated with the number of events and subjects with event for each MedDRA SOC/preferred term and overall. Rates will be reported as the number of subjects who

experience at least one event out of the total number of subjects. Serious adverse events will be summarized in the same way.

Device deficiencies will be summarized by type of device observation in the safety population.

Subject deaths will be provided in listing format.

6.9 Subgroup Analyses

Primary efficacy will be summarized for the following subgroups: HCA (Y/N), IV contrast within 48hrs prior to surgery (Y/N), biological sex (M/F), age (Age<65, Age≥65), baseline diabetes mellitus (Y/N), baseline heart failure (Y/N), and baseline hypertension (Y/N).

The outcome of interest will be AKI severity of 0 versus AKI severity of 1/2/3 at 48 hours. Logistic regression will be used to determine the odds ratio for SoC+ CytoSorb versus SoC within each subgroup. The odds ratio and associated 95% confidence intervals for each subgroup will be presented in a forest plot. The analysis will be repeated but with the outcome being AKI severity of 0 or 1 versus AKI severity of 2 or 3.

Analyses will be performed in the ITT population (excluding Roll-ins). These analyses are intended to demonstrate consistency of results across subgroups.

6.10 Protocol Deviations

A protocol deviation is a failure to comply with the requirements specified within the clinical study protocol.

The protocol deviations for this study consist of, but not limited to the following:

- Failure to obtain subject's informed consent prior to any study-related activities
- Failure to conduct protocol required clinical follow-ups
- Failure to conduct protocol required clinical follow-ups within time windows
- Failure to report serious adverse events according to protocol requirements.

Protocol deviations will be classified as either minor or major. A major protocol deviation is any deviation that could impact subject safety or the integrity of the trial.

The sponsor, or designee, will be responsible for producing the final protocol deviation file (formatted as a Microsoft Excel file) with major/minor classification, which will be finalized prior to database lock. This file will include site, subject ID, deviation date, deviation category, deviation type (major/minor), and a description of the protocol deviation.

The number and percentage of treated subjects with major protocol deviations will be summarized by deviation category, treatment group and overall. Deviation types will be presented in alphabetical order.

7 Changes from Planned Analyses

Any changes to planned statistical analyses determined necessary prior to performing the analyses will be documented in an amended Statistical Analysis Plan and approved prior to the analysis when possible. Any other deviations or changes from the planned analyses deemed necessary due to violation of critical underlying statistical assumptions, data characteristics, or missing data will be clearly described in the clinical study report with justification and rationale.

8 References

Mehta CR, Pocock SJ. Adaptive increase in sample size when interim results are promising: a practical guide with examples. *Stat Med.* Dec 10 2011;30(28):3267-3284.

Chen YH, DeMets DL, Lan KK. Increasing the sample size when the unblinded interim result is promising. *Stat Med.* Apr 15 2004;23(7):1023-1038.

REFRESH II SAP

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